Amendment to the Specification:

Please amend the specification as follows:

In the Cross-Reference to Related Applications on page 1:

--This application is a continuation of [[the US national stage designation of]] International application[[PCT/IL99/00851]] PCT/IL99/00581 filed November 2, 1999, the content of which is expressly incorporated herein by reference thereto.

Page 7, last full paragraph:

-- Seven consecutive amino acids of the CDR3 loop form a structural mimic of the natural carbohydrate substrate of the enzyme. Therefore, the exposed CDR3 loop of the dromedary VH in that disclosure, or generally VH domains that will be isolated from libraries, might be good candidates to serve as a lead compound for new drugs. Another example is the selection of a camelized VH domain that acts as an inhibitor of hepatitis C virus NS3 protease (Martin et al. Protein Eng. 10,607-614,1997). Another example arises from ~~our findings herein that we were able to isolate~~ a VH protein that was isolated that can bind specifically Ig of different types and species. This product can be further developed as a specific reagent for detection, purification, and analysis of antibodies. This can be performed on the intact VH protein or alternatively using the CDR3 encoded peptide that is responsible for the unique binding specificity. --

Page 19, first paragraph under "Results" heading:

-- In most VH families residue 44 is a Glycine, ~~we screened initially our~~ the cloned VH genes were screened for families in which position 44 is other than Glycine and [[selected]] a VH was selected that was cloned from a mouse hybridoma generated against an HIV peptide in complex with H-2Dd. The VH belongs to mouse VH group I (A), the nucleotide and amino acid sequence are presented in Figure 1. --

Page 20, first paragraph under "Results" heading:

-- Examples for panning on two antigens, Tumor necrosis factor alpha (TNF) and Ig are shown in Table 1. Soluble recombinant TNF immobilized to sulfated polystyrene latex beads was subjected to four rounds of panning. The number of phage captured on the antigen-

coated beads increased by more than 80-fold with the fourth round of panning. Forty individual phage clones from the fourth round of panning were tested in a phage ELISA assay for binding to immobilized TNF. Positive clones were sequenced. Example for phage ELISA results using individual clones are presented in Figure 3A. ~~We were able to isolate~~ Isolated clones [[which]] were strongly positive and specific for TNF.--

Paragraph bridging pages 20-21:

-- The VH library was also used in a panning experiment in which biotinylated IgG was immobilized on Streptavidine-coated magnetic beads and Ig binding phage clones were isolated. As shown in Table 1 B, after four rounds of panning a 150-fold enrichment in the number of phage captured by antigen was observed. Phage ELISA of individual clones revealed strong and specific binding of the antigen compared to control phage (Figure 3B). The genes encoding the VH protein were rescued from positive phage clones and their sequences were analyzed. As shown in Table 2, all clones exhibited an intact VH insert that contained a random 9 amino acid stretch at the expected location of CDR3. Sequence analysis revealed also consensus residues between positive clones that were isolated after the forth round of panning. For example, clones number 1 and 4 which recognize Ig show a consensus sequence of GLY-X-SER-PRO-GLN. It may be noted that in these cases X is a hydrophilic residue though this may not be a straight requirement. The difference is the location of the consensus within the CDR. [[For]] Clone 4 was chosen for a detailed characterization of a VH single domain protein ~~we choose to use clone~~ 4. Consensus sequences were also obtained in several independent screenings in which antigens were immobilized on polystyrene latex beads and binding phage clones were characterized and found to be specific for plastic (polystyrene). This phenomena is characterized in the literature using peptide phage display libraries and consensus sequences rich in Trp and Tyr, which bind plastic (Adey et al. ibid). Several VH phage clones with such consensus sequences were isolated as shown in Table 2. These results demonstrate that individual antigen-binding phage clones can be isolated from the VH library. These phage clones are highly reactive in phage ELISA assays and are specific for the antigen. DNA sequence analysis of the clones isolated after the fourth round of panning revealed that the enrichment was specific for individual clones, thus, 50-60% of the sequences obtained were identical at the expected region of CDR3. --

Page 22-23, paragraph under "Results" heading bridging those pages:

--To produce soluble VH protein, ~~we have rescued~~ the VH gene was rescued from the isolated phage genome by PCR and was subcloned [[the gene]] into a pET system expression vector in which expression is driven by the T7 promoter. Expression of the VH genes in E Coli BL21 cells was very efficient and recombinant protein accumulated as insoluble intracellular inclusion bodies. The VH could be detected as the major band on SDS/PAGE of solubilized whole cell as well as isolated purified inclusion bodies. Purified inclusion bodies contained >90% recombinant VH protein. Although expression and production of VH was very efficient for all VH genes that were isolated (from TNF and Ig phage clones), ~~we choose to~~ focus was centered on the characterization of one VH protein from phage clone #1 that recognize Ig. Inclusion bodies were purified, solubilized in 6M guanidine HCI, and refolded by in-vitro redox-shuffling buffer system. --

Page 24, first paragraph under "Results" heading:

--The major concern with VH single domain phage is the non specific binding due to their exposed VL interface. The reduced area of the potential antigen binding site in an isolated VH, compared with a combination of VH and VL, might also compromise specificity. ~~We therefore analyzed by phage ELISA assays the~~ The binding of the isolated phage clones to various control antigens was analyzed by phage ELISA assays. As shown in Figure 3C the isolated VH single domain phage that recognize IgG proved to be highly specific. No binding to any antigen other than that selected on, was detected. Similar specificity studies were performed on the phage clones that recognize TNF and similar results were obtained (Figure 4). The phage clones that were isolated by panning on IgG recognized specifically a range of immunoglobulins from different species including hamster, human, mouse, and rabbit IgG. They also recognize different Ig isotypes such as IgM, IgG1, IgG2a and IgG2b. Similar results were obtained when soluble VH single-domain protein was made from the periplasm of phage clones 1 and 4. These results suggest phage clones isolated form the VH library can bind very specifically to the antigen to which they were selected for and they are not sticky. Analysis of the binding characteristics of soluble, purified VH proteins that were generated from the isolated phage clones further indicate the specificity results that were obtained with the parental phage clones. --

Paragraph bridging pages 26-27:

--The results suggest that the VH domain is folded in the correct conformation and is similar to other Ig domains with known CD spectra. To clearly demonstrate that the VH protein is a predominantly monomeric, ~~we performed~~ analytical ultracentrifugation was performed. The sedimentation equilibrium profiles of the VH protein were measured over a 50-fold range of protein loading concentrations and at 3 different rotor speeds. These profiles could not be satisfactory globally fitted on the basis of a single monomeric species with the molar mass and partial specific volume predicted by the amino acid composition. Independent analysis of the sedimentation profiles at the highest and lowest loading concentrations with the floating molar mass led to a best-fit molar mass increasing with concentration. This indicated that the protein exhibited a weak self-association. Global analysis of all the equilibrium data using the predicted buoyant molar mass and considering reversible dimerization resulted in a fit of high quality, with a best-fit association constant (Ka) for dimer formation of 900 i 100 M-l. This result indicates that the VH protein has a very low weak tendency of dimerization. Sedimentation velocity experiments were well-described by the Lamm-equation model for rapid monomer-dimer equilibria, with a best-fit monomer sedimentation coefficient of 1.7S. These results also correlate well with dynamic light scattering data, which lead to a hydrodynamic radius of 2.0 nm. No indications of higher aggregates were found in sedimentation equilibrium, sedimentation velocity, and light scattering experiments. The association constant obtained corresponds to a dissociation equilibrium constant 1.1 mM. This would suggest that at a concentration of 1 mM (-17 mg/ml) 50% of the VH protein is in the form of a dimer and 50% in the form of a monomer. These results are the first demonstration of such biophysical analysis of a VH protein using analytical ultracentrifugation. --

Page 28, first paragraph under "Results" heading:

--To determine the binding properties of the purified VH protein ~~we performed~~ several studies were performed in which assay the binding to antigen directly or by a secondary reagent in an indirect test. First, an ELISA assay was performed to titrate the binding of the VH protein to human IgG which is immobilized onto maxisorb ELISA plates. This is an indirect assay due to the fact that binding is being monitored by a secondary peroxidase-labeled antibody directed to the E-tag sequence at the carboxy terminus of the VH

protein. As shown in Figure 7A the VH binds human IgG in a dose dependent manner and VH protein concentrations as low as 1.7 ng/ml (100 pM) could be detected. When tested for specificity, the purified VH protein recognized a large variety of Ig's from different species and different isotypes. The results demonstrate that the VH recognizes specifically Ig's and that this recognition lies in the CH1 or CL domains since the VH protein recognized a Fab fragment but not an Fv fragment (Figure 8A). No binding was detected on control antigens. To determine the binding affinity of the purified VH protein to its antigen, ~~we performed~~ two types of binding assays were performed. First, ~~we performed~~ a competition binding analysis was performed with radiolabeled VH protein and second using real-time surface plasmon resonance (SPR) technology. In the competition binding analysis the iodinated VH protein was used as a tracer with increasing concentrations of competing unlabeled purified VH and tested for binding to immobilized human. Apparent binding affinity was-100 nM at which 50% inhibition of binding of iodinated VH protein had occurred (Figure 7B). For the real-time surface plasmon resonance measurements, ~~we efficiently coupled~~ a mouse IgG monoclonal antibody was efficiently coupled to the dextran matrix of the biosensor by random coupling through free amino groups. To estimate the kinetic association and dissociation rate constants of the VH protein for the IgG, ~~we injected~~ homogeneously loaded, highly purified VH single-domain protein at different concentrations were injected over an IgG surface (Figure 8B). By curve fitting to the dissociation (washout) phase of the binding curve, ~~we determined~~ the kinetic dissociation rate constant was determined to be kd = 4.13 x10-3 s-l corresponding to a tl/2 of 168 s. The association rate constant was determined to be ka =2.14x105 M-ls-l. These values give a calculated equilibrium constant for dissociation, Kd = kd/ka of 1.9 x 10-8 M. These results correlate with the results obtained in the competition binding assay and indicate that the VH protein has a good binding affinity to its IgG antigen.--